

**IN THE CLAIMS:**

1. **(Currently Amended)** An isolated nucleic acid fragment ~~encoding a nitrilase enzyme~~ selected from the group consisting of:

- (a) an isolated nucleic acid fragment encoding a polypeptide with nitrilase activity ~~all or a substantial portion of the amino acid sequence~~ selected from the group consisting of SEQ ID NO:5 and SEQ ID NO:14;
- (b) ~~an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence selected from the group consisting of SEQ ID NO:5 and SEQ ID NO:14;~~
- (e) an isolated nucleic acid molecule that hybridizes with the isolated nucleic acid fragment of (a) under hybridization conditions of 6X SSC (1M NaCl), 40 to 45 % formamide, 1 % SDS at 37 °C, and a wash in 0.5X to 1X SSC at 55 to 60 °C; and
- (d) (c) an isolated nucleic acid fragment that is completely complementary to (a) ; or (b) ~~or (e)~~ .

2. **(Currently Amended)** An isolated nucleic acid fragment comprising a first nucleotide sequence encoding a polypeptide with nitrilase activity ~~of at least 369 amino acids~~ that has greater than 71 % identity based on the Needleman and Wunsch algorithm when compared to a polypeptide encoded by the sequence identified in SEQ ID NO:5, or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

3. **(Currently Amended)** An isolated nucleic acid fragment encoding a nitrilase enzyme, ~~or a fragment thereof~~, selected from the group consisting of:

- (a) an isolated nucleic acid fragment selected from the group consisting of ~~SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15 and SEQ ID NO:16;~~
- (b) an isolated nucleic acid molecule that hybridizes with the isolated nucleic acid fragment of (a) under hybridization conditions of 6X SSC (1M NaCl), 40 to 45 % formamide, 1 % SDS at 37 °C, and a wash in 0.5X to 1X SSC at 55 to 60 °C; and
- (c) an isolated nucleic acid fragment that is completely complementary to (a) or (b).

4. **(Original)** An isolated nucleic acid sequence encoding a nitrilase enzyme selected from the group consisting of SEQ ID NO:4, SEQ ID NO:13, SEQ ID NO:15, and SEQ ID NO:16.

5. **(Original)** The isolated nucleic acid fragment of any of Claims 1, 2, 3, or 4 wherein the fragment is isolated from an *Acidovorax* strain.

6. **(Withdrawn)** A polypeptide encoded by the nucleic acid fragments of any of Claims 1, 2, 3, or 4.

7. **(Withdrawn)** A polypeptide according to Claim 6 having the amino acid sequence selected from the group consisting of SEQ ID NO:5 and SEQ ID NO:14.

8. **(Withdrawn)** The polypeptide of Claim 6 further characterized by nitrilase activity on nitrile-containing substrates selected from the group consisting of aliphatic nitriles and aromatic nitriles.

A.2 9. **(Currently Amended)** A chimeric gene comprising the isolated nucleic acid fragment of any of Claims 1, 2, 3, or 4 or 5 operably linked to suitable regulatory sequences.

10. **(Original)** A plasmid pSW91 contained in *E. coli* SW91 having the designation ATCC PTA-1175, a plasmid pnit4 contained in *E. coli* DH5  $\alpha$ : pnit4 having the designation ATCC PTA-1176, or a plasmid pnitex2 contained in either *E. coli* SS1002 or in *E. coli* SS1011.

11. **(Original)** An expression cassette comprising the chimeric gene of Claim 9.

12. **(Original)** The expression cassette of Claim 11 selected from the group consisting of the plasmids pSW91, pnit4, and pnitex2.

13. **(Original)** A transformed microorganism comprising the chimeric gene Claim 9.

14. **(Original)** A transformed microorganism comprising the plasmid of Claim 10.

15. **(Original)** A transformed microorganism comprising the expression cassette of Claim 11.

16. **(Original)** The transformed microorganism of Claim 15 wherein the expression cassette is chromosomally integrated.

17. **(Original)** The transformed microorganism of Claim 16 further comprising suitable regulatory sequences.

18. **(Original)** The transformed microorganism of Claim 17 wherein the suitable regulatory sequences comprise

- a) at least one promoter selected from the group consisting of the tryptophan operon promoter P<sub>trp</sub> of *E. coli*, a lactose operon promoter Plac of *E. coli*, a P<sub>tac</sub> promoter of *E. coli*, a phage lambda right promoter P<sub>R</sub>, a phage lambda left promoter P<sub>L</sub>, a T7 promoter, a promoter of the AOX1 gene from *Pichia pastoris*, and a promoter of the GAP gene from *Pichia pastoris*, or is at least one strong promoter selected from the group consisting of *Comamonas*, *Corynebacterium*, *Brevibacterium*, *Rhodococcus*, *Azotobacter*, *Citrobacter*, *Enterobacter*, *Clostridium*, *Klebsiella*, *Salmonella*, *Lactobacillus*, *Aspergillus*, *Saccharomyces*, *Pichia*, *Zygosaccharomyces*, *Kluyveromyces*, *Candida*, *Hansenula*, *Dunaliella*, *Debaryomyces*, *Mucor*, *Torulopsis*, *Methylobacteria*, *Bacillus*, *Escherichia*, *Pseudomonas*, *Rhizobium*, and *Streptomyces*, and
- b) at least one ribosome binding site from a phage lambda CII gene or selected from the group consisting of ribosome binding sites from a gene of *Comamonas*, *Corynebacterium*, *Brevibacterium*, *Rhodococcus*, *Azotobacter*, *Citrobacter*, *Enterobacter*, *Clostridium*, *Klebsiella*, *Salmonella*, *Lactobacillus*, *Aspergillus*, *Saccharomyces*, *Zygosaccharomyces*, *Pichia*, *Kluyveromyces*, *Candida*, *Hansenula*, *Dunaliella*, *Debaryomyces*, *Mucor*, *Torulopsis*, *Methylobacteria*, *Bacillus*, *Escherichia*, *Pseudomonas*, *Rhizobium*, and *Streptomyces*.

19. **(Original)** The transformed microorganism of Claim 18, wherein the host microorganism is selected from the group consisting of *Comamonas*, *Corynebacterium*, *Brevibacterium*, *Rhodococcus*, *Azotobacter*, *Citrobacter*, *Enterobacter*, *Clostridium*, *Klebsiella*, *Salmonella*, *Lactobacillus*, *Aspergillus*, *Saccharomyces*, *Zygosaccharomyces*, *Pichia*, *Kluyveromyces*, *Candida*, *Hansenula*, *Dunaliella*, *Debaryomyces*, *Mucor*, *Torulopsis*, *Methylobacteria*, *Bacillus*, *Escherichia*, *Pseudomonas*, *Rhizobium*, and *Streptomyces*.

20. **(Original)** A transformed microorganism selected from the group consisting of:

- (a) *E. coli* SW91 having the designation ATCC PTA-1175;
- (b) *E. coli* DH5 $\alpha$ : pnit4 having the designation ATCC PTA-1176;
- (c) *E. coli* SS1001 having the designation ATCC PTA-1177; and
- (d) *E. coli* SS1002 containing plasmid pnitex2; and
- (e) *E. coli* SS1011 containing plasmid pnitex2.

21. **(Cancelled)** A method of obtaining a nucleic acid fragment encoding all or a substantial portion of a nitrilase enzyme, the method comprising:

- (a) probing a genomic library with all or a portion of a nucleic acid fragment selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, and SEQ ID NO:16;
- (b) identifying a DNA clone that hybridizes with the nucleic acid fragment of step (a); and
- (c) sequencing the nucleic acid fragment that comprises the DNA clone identified in step (b),

wherein the sequenced nucleic acid fragment of step (b) encodes all or a substantial portion of an amino acid sequence encoding a nitrilase enzyme.

22. **(Cancelled)** A method of obtaining a nucleic acid fragment encoding all or a substantial portion of a nitrilase enzyme, the method comprising:

- (a) synthesizing at least one oligonucleotide primer corresponding to a portion of the sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, and SEQ ID NO:16; and
- (b) amplifying an insert present in a cloning vector using the oligonucleotide primer of step (a), the amplified insert of step (b) encoding all or a substantial portion of an amino acid sequence encoding a nitrilase enzyme.

23. **(Cancelled)** The product of the method of Claims 21 or 22.  
Claims 24. through 45. **(Withdrawn)**

46. **(Original)** The transformed microorganism of Claim 19, wherein the host microorganism is *E. coli* strains MG1655 (ATCC 47076), W3110 (ATCC 27325), MC4100 (ATCC 35695), or W1485 (ATCC 12435).

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A<sup>3</sup> 47. **(New)** An isolated nucleic acid fragment encoding a polypeptide having the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:14.

48. **(New)** A chimeric gene comprising the isolated nucleic acid fragment of Claim 5 operably linked to suitable regulatory sequences.

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